May 18, 1998

Dr. C.W. Jameson
National Toxicology Program
Report on Carcinogens, MD EC-14
P.O. Box 12233
Research Triangle Park, N.C. 27709

Dear Dr. Jameson,

Enclosed find comments from the National Soft Drink association regarding the announcement in the Federal Register, Vol. 53, March 19, 1998 pertaining to the Report on Carcinogens, Ninth Edition. I am both faxing, as well as mailing, a copy to you. Thank you for the opportunity to comment on this report.

Sincerely,

Richard H. Adamson

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NATIONAL TOXICOLOGY PROGRAM CALL FOR PUBLIC COMMENTS: AGENTS, SUBSTANCES, MIXTURES AND EXPOSURE CIRCUMSTANCES PROPOSED FOR LISTING IN OR REMOVING FROM THE REPORT ON CARCINOGENS, NINTH EDITION

FEDERAL REGISTER VOL. 53
MARCH 19, 1998

COMMENTS OF
THE NATIONAL SOFT DRINK ASSOCIATION

TO THE
NATIONAL TOXICOLOGY PROGRAM

MAY 18, 1998

Introduction

The National Soft Drink Association (NSDA) is pleased to submit comments in response to the National Toxicology Program solicitation of public comments on its intent to recommend additional agents, substances, mixtures and exposure circumstances for listing in or delisting from the Report on Carcinogens, Ninth Edition. This report is to contain a list of all substances (1) which either are known to be human carcinogens or may reasonably be anticipated to be human carcinogens; (emphasis added) and (2) to which a significant number of persons residing in the United States are exposed. NSDA is submitting comments on saccharin, one of the fourteen substances listed in the Federal Register notice (Federal Register vol. 63, no. 53, March 19, 1998 pp. 13418-13420). As more fully set forth below, NSDA supports delisting saccharin as a human carcinogen and as an agent reasonably anticipated to be a human carcinogen.

NSDA is the national trade organization of the United States soft drink industry. NSDA's members produce more than 90% of all soft drinks consumed annually in the United States. In addition, the vast majority of soft drink licensors who manufacture the concentrates and/or syrups from which soft drinks are made, belong to the Association. It is on behalf of these members that we submit these comments.

Comments

NSDA believes that when consideration is given to all relevant information available on saccharin in the peer reviewed literature, saccharin cannot be "known to be a human carcinogen" nor "reasonably anticipated to be a human carcinogen". Therefore, NSDA supports the petition to delist saccharin from the Report on Carcinogens, Ninth Edition.

NSDA summarizes below peer reviewed data, albeit briefly, on the structure of saccharin and its metabolism, genotoxic studies, animal bioassays, epidemiological studies and mechanistic studies.

Structure

Saccharin chemically is 1,2-Benzisothiazol-3(2H)-one 1,1 dioxide. There are several forms available including acid saccharin, sodium saccharin, potassium saccharin and calcium saccharin. Some of the biological effects of saccharin are dependent on the chemical form and, therefore, the form of the compound needs to be noted when discussing its effects. It is important to note that the saccharin molecule (anion) is a nucleophile and therefore would not be expected to be a genotoxic carcinogen nor would it form DNA adducts unless it was metabolized to an electrophile.

Metabolism

Results from many studies in several species including rodents, rhesus monkeys and humans have generally shown that following administration, sodium saccharin is excreted unchanged. The majority of the compound is excreted in the urine and a smaller percentage in the feces (IARC, 1980; Renwick 1985, Whysner and Williams, 1996). Identification of radioactivity in the urine of rats following administration of ^{14}C or ^{35}S labeled sodium saccharin was found to be >99% unmetabolized saccharin. Also, studies have shown that no metabolites of saccharin were found in urine even following pretreatment with phenobarbital or sodium saccharin. Therefore, metabolism of saccharin to an electrophile that is DNA reactive, is not known and highly unlikely. Finally, as discussed by Renwick, the disposition of sodium saccharin in the rat at dietary levels of 5% or greater is not representative of its disposition at

lower doses. At these higher levels, there is extensive retention of saccharin in the plasma and tissues and saturation of renal tubular secretion (Renwick, 1985).

Genotoxic Studies

Following administration of ^{35}S-labeled sodium saccharin (Lutz and Schlatter,1977), it was shown that there was no binding to the DNA of rat liver or urinary bladder. In another study (Miyata et. al., 1980), it was found that sodium saccharin did not damage DNA in rat bladder epithelium with or without preexposure to a genotoxic bladder carcinogen. An in vitro study with acid saccharin using cultures of rat hepatocytes showed a higher rate of DNA single strand breaks, but only at the highest dose (Sina et. al. 1983).

Most tests for mutagenicity of sodium saccharin or acid saccharin in various tester strains of Salmonella typhimurium have been negative. Also, cell transformation tests with (mouse C3H/1OT ½) sodium saccharin or acid saccharin are negative. Thus, there is little evidence that sodium saccharin induces point mutations. There are some positive genotoxic tests for sodium saccharin but the end point in most of the positive experiments is chromosomal breaks and aberrations, not point mutations. Furthermore, other sodium and potassium salts (e.g. NaCl and KCl) are also positive in some of these studies at similarly high doses. The genotoxicity studies have been reviewed by both IARC (IARC, 1980, 1987) and Whysner and Williams (1996).

Animal Bioassays

The studies of the carcinogenic potential of saccharin following administration to animals (animal bioassays) has been summarized and critically evaluated by IARC (1980, 1987), the Office of Technology Assessment (1977) and more recently by Whysner and Williams (1996). A brief summary of the results of these bioassays in various species is listed below.

Mice In studies that could be evaluated, oral administration of saccharin did not cause tumors in mice in either single or multi-generation studies (IARC 1980, 1987). The study of the mixture of sodium saccharin with cholesterol (1:4 by weight), followed by insertion of a pellet into the urinary bladder lumina in mice gave positive results with the mixture as compared with cholesterol pellets per se (Bryan, et. al 1970). Not only is this design unphysiologic, but it is essentially an artifact of the laboratory and the tumors are due to the pellet per se, not the compound mixed with the cholesterol (Jull, 1979: Clayson et. al., 1995). Thus, well designed and conducted studies of sodium saccharin administration to mice do not give evidence of carcinogenicity.

Rats Both acid saccharin and sodium saccharin have been bioassayed in rats for potential carcinogenicity in one generation studies. A summary of sixteen different studies of administration of acid saccharin or sodium saccharin in one generation studies showed that statistically significant bladder tumors were induced in only one study (Whysner and Williams, 1996; Fukushima et. al. 1983). Also, in this study in ACI rats there was a difference between the sodium saccharin treated and the control rats in that the treated rats (more than 50% and perhaps 100%) were infected with the bladder parasite Trichosomoides crassicauda, which may have enhanced cellular proliferation. Only in two generation feeding studies to rats (from the moment of conception until death) at higher doses has sodium saccharin consistently produced bladder cancer. More bladder cancers were found in male than females and the dose that produced these cancers was generally at 5.0 or 7.5% in the diet (IARC, 1980, 1987; Office of Technical Assessment, 1977; Whysner and Williams, 1996). In these studies, there was dose related hyperplasia, and dose related tumors of the bladder. However, changes in urine volume and

osmolidity were highly correlated with the occurrence of urinary bladder tumors (see discussion under mechanistic studies). It is important to reiterate, as discussed under metabolism, that the disposition of sodium saccharin, at these high dietary levels, is not representative of its disposition at lower levels.

Hamsters A study of sodium saccharin in Syrian golden hamsters, at doses up to 1.25% in drinking water for life, was reported by Althoff et. al (1975). The average daily consumption of saccharin ranged from 44mg/animal (at the 0.156% dose) to 353 mg/animal (at the 1.25% dose). No difference in tumors was seen in controls and treated animals.

Non-human primates Two studies have been reported in non-human primates. In one study, sodium saccharin was administered orally at doses of 20, 100 or 500 mg/kg daily for 6 days/week to 2, 2 and 3 rhesus monkeys of each sex and the animals were autopsied at 79 months following treatment. Three male and three female monkeys served as controls. No abnormal pathology was found in urinary bladder, kidney or testes in the 12 monkeys in the treated group which were remaining at autopsy (Coulston, et. al., 1975; McChesney,et. al. 1977). In the second study, sodium saccharin was administered to six African green, seven rhesus and six cynomolgus monkeys at a dose of 25 mg/kg beginning 24 hours after birth for up to 24 years. The authors have argued that the rhesus and cynomolgus monkeys can be treated as a group and perhaps also the African green monkeys. During the last two years of life, urine was collected from selected treated and control animals and evaluated for the presence of calculi, microcrystalluria and precipitate. The urinary bladders were examined by both light and scanning electron microcopy. Results showed that sodium saccharin did not cause bladder cancer in any of the monkeys. There also was no effect on the urine or urothelium in any of the

monkeys and there was no evidence of increased urothelial cell proliferation or formation of solid material in the urine (Takayama et. al., 1998). There were benign tumors in two of the sodium saccharin treated monkeys and a lymphoma of the thyroid in one of the treated monkeys. These tumors have also been found in normal controls and breeders in this colony. Both of these studies, although not definitive, provide additional evidence that sodium saccharin is without a carcinogenic effect on the primate urinary tract.

Epidemiological Studies

The epidemiological studies have been summarized by IARC (1980, 1987), Whysner and Williams (1996) and Silverman et. al. (1996). Results of most case control studies have been negative for the association of artificial sweetener use and bladder cancer. Fourteen case control studies are available in the scientific literature and only one suggested a positive association. In this study in men, there was an increase in relative risk, but there was an inverse association in women (Howe et. al., 1977; Silverman et. al., 1996). In a large population based case control study in the U.S., there was no elevation in relative risk for subjects who reported ever having used artificial sweeteners (Hoover and Strasser, 1980). The reasons for a positive association seen in two subgroups, white heavy smokers and nonsmoking white females, is uncertain but may or may not reflect biological reality. The increased relative risk in these subgroups has not been seen in other large studies (Risch et. al., 1988). Also, bladder cancer among a population in Denmark born during World War II years, a group with higher in utero saccharin exposure than previous birth cohorts, was not increased in either sex during the three decades of life (Jensen and Kamby, 1982). Finally, a meta-analysis of several of the case control studies on artificial sweeteners and bladder cancer resulted in a relative risk of 0.97 (Elcock and Morgan, 1993).

Therefore, epidemiological studies indicate that the use of saccharin is associated with little or no excess risk of bladder cancer.

Mechanistic Studies

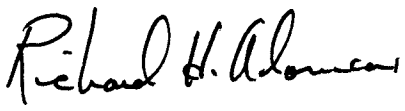
The induction of tumors in rats following administration of sodium saccharin in the diet, in two generation studies, but not in mice, hamsters or nonhuman primates and with no substantial evidence in humans, has prompted numerous studies on the mechanism(s) responsible for the effect. The induction of bladder tumors, which occurs primarily in the male rat, occurs at high doses of dietary sodium saccharin which also increases urothelial cell proliferation. This proliferation is likely regenerative secondary to cytotoxicity of the superficial cells of the urothelium (Cohen and Ellwein 1990; Cohen et. al., 1995). As has been summarized, several changes in the urine are necessary for proliferative and tumorigenic activity of saccharin in the rat (Whysner and Williams, 1996; Cohen et. al., 1995). These include the chemical form of saccharin, i.e. the sodium salt is important; the presence of a urinary pH of 6.5 or greater; increased urinary volume, high urinary protein, especially the low molecular weight protein $\propto 2\mu$ globulin and the diet. Evidence is also accumulating that an amorphous precipitate occurs in the urine of male rats following high doses of various sodium salts which may be related to the urothelial effects by causing mechanical irritation. These multiple variables in rat urine, critical to development of bladder cancer in this species following high dose sodium saccharin, do not occur in humans and thus no effect in the human urinary tract should occur.

Summary and Conclusions

Saccharin has a nucleophilic structure and it is not activated to an electrophile. There is no indication that saccharin binds to DNA, it does not induce point mutations and it is not

carcinogenic in mice, hamsters or non-human primates. It is carcinogenic in two generation studies at high dietary doses in rats. Mechanistic studies strongly indicate that this is a high dose, species-specific effect due to cytotoxicity and cellular proliferation in the bladder. Epidemiological studies demonstrate little or no excess risk of human bladder cancer following consumption of saccharin. Therefore, saccharin is not known to be a human carcinogen nor can it reasonably be anticipated to be a human carcinogen and should be delisted in the Report on Carcinogens, Ninth Edition.

Respectfully submitted,



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